Appendix A; Response Mailed On 01/16/02
Unmarked Claims

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1. (Amended) An enzyme-linked in-*situ* hybridization probe further characterized in that it comprises a probing nucleobase sequence that specifically hybridizes to a yeast specific target sequence.

2. (Restated) The probe of claim 1, wherein the target sequence is ribosomal RNA.

3. (Restated) The probe of claim 1, wherein the probe is a nucleic acid.

4. (Restated) The probe of claim 1, wherein the probe is a peptide nucleic acid.

5. (Amended) The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate organisms of one or more species of yeast.

6. (Amended) The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate organisms of one or more genus of a yeast.

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7. (Amended) The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate all yeast in a sample.

8. (Restated) The probe of claim 1, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

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9. (Amended) An enzyme-linked probe for detecting, identifying or quantitating the presence of *Dekkera/Brettanomyces* yeast in a sample of interest.

10. (Restated) The probe of claim 9, wherein the probe comprises a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA

(Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

11. (Amended) The probe of claim 10, wherein the probing nucleobase sequence is selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

12. (Restated) The probe of claim 9, wherein the probe is a peptide nucleic acid.

16. (Restated) The probe of claim 15, wherein the probe is labeled with soy-bean peroxidase.

18. (Restated) The probe of claim 9, wherein the probe is support bound.

19. (Restated) The probe of claim 18, wherein the probe exists attached to an array of probes.

20. (Amended) A set of enzyme-linked probes for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample of interest.

21. (Amended) The probe set of claim 20, wherein one or more of the probes comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

22. (Amended) The probe set of claim 21, wherein the probing nucleobase sequences of said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

b) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample.

47. (Restated) The method of claim 46 further comprising the step of:

c) isolating the yeast using a filter as an isolation medium.

48. (Amended) The method of claim 47, further comprising the step of:

d) growing the isolated yeast by culture in media.

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49. (Amended) The method of claim 48, wherein the culture is grown directly on the filter, under suitable culture conditions, by placing the filter in contact with media.

60. (Amended) A method for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample; said method comprising:

a) contacting one or more species of yeast in the sample with one or more enzyme-linked *Dekkera/Brettanomyces* yeast specific probes, under suitable hybridization conditions, to thereby form a probe/target sequence hybrid; and

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b) detecting the presence, absence or amount of probe/target sequence hybrid and correlating the result with the presence, absence or number of *Dekkera/Brettanomyces* yeast in the sample.

Sub C11

61. (Amended) The method of claim 60, wherein one or more of the *Dekkera/Brettanomyces* yeast specific probes comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

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62. (Amended) The method of claim 61, wherein the probing nucleobase sequences of said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

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72. (Amended) A kit for performing an assay that detects, identifies or enumerates *Dekkera/Brettanomyces* yeast in a sample, wherein said kit comprises:

a) one or more enzyme-linked *Dekkera/Brettanomyces* specific probes; and

b) other reagents or compositions necessary to perform the assay.

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80. (Amended) The kit of claim 72, comprising:

a) a filter for isolating yeast from a sample of interest;

b) culture media for growing the isolated yeast;

c) fixation solution for fixing grown yeast;

d) a hybridization solution suitable for imposing suitable hybridization conditions;

e) a soy bean peroxidase enzyme labeled probe specific for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in the sample;

f) one or more wash solutions for removing undesirable components after performing one or more steps of the assay; and optionally

g) an enzyme substrate suitable for generating detectable signal from [the] enzyme activity of the soy bean peroxidase enzyme linked to the [peptide nucleic acid] probe; or

h) a film for detecting signal generated from the enzyme activity.

81. (Restated) The kit of claim 80, wherein the fixation solution and the hybridization solution are the same solution.

82. (Restated) The kit of claim 80, wherein the soy bean peroxidase labeled probe is a peptide nucleic acid.

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83. (Amended) A method for quantitating slow growing yeast in a liquid sample in less than 48 hours; said method comprising:

a) filtering a fixed volume of liquid using a filter having a pore size that does not allow the yeast to pass;

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b) incubating the filter containing the yeast, in media and under culture conditions, for 45 or fewer hours to thereby grow microcolonies of yeast;

c) fixing the microcolonies of yeast to the filter;

d) contacting the microcolonies of yeast with a yeast specific enzyme-linked probe, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast;

e) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample; and

f) determining the quantity of yeast in the sample.

84. (Amended) The method of claim 83, wherein fixing the microcolonies of yeast to the filter and contacting the microcolonies of yeast with a yeast specific enzyme-linked probe are performed simultaneously using a single solution.

85. (Amended) The method of claim 83, wherein the number of CFU in the sample is determined.

Sub C13

86. (New) The probe set of claim 9, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

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87. (New) The probe set of claim 20, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

III. AMENDMENT

(i) *Please cancel, without prejudice, claims 13-15, 17, 27, 28, 30 and 31.*

(ii) *Please amend the claims as follows:*

1. (Amended) An enzyme-linked in-*situ* hybridization probe [suitable for use in an *in-situ* hybridization assay and] further characterized in that it comprises a probing nucleobase sequence [directed] that specifically hybridizes to a yeast specific target sequence.

5. (Amended) The probe of claim 1, wherein the probing nucleobase sequence is [designed] selected to detect, identify or enumerate organisms of one or more species of yeast.

6. (Amended) The probe of claim 1, wherein the probing nucleobase sequence is [designed] selected to detect, identify or enumerate organisms of one or more genus of a yeast.

7. (Amended) The probe of claim 1, wherein the probing nucleobase sequence is [designed] selected to detect, identify or enumerate all yeast in a sample.

9. (Amended) An enzyme-linked probe [suitable] for detecting, identifying or quantitating the presence of *Dekkera/Brettanomyces* yeast [and particularly *Dekkera bruxellensis (Brettanomyces)*] in a sample of interest.

11. (Amended) The probe of claim 10, wherein the probing nucleobase sequence is [exactly as it appears] selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

20. (Amended) A set of enzyme-linked probes [set suitable] for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample of interest.

21. (Amended) The probe set of claim 20, wherein one or more of the probes [specific for *Dekkera/Brettanomyces* yeast] comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID

No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

22. (Amended) The probe set of claim 21, wherein the probing nucleobase sequences [are exactly as represented] <u>of said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified</u> in the claim.

23. (Amended) The probe set of claim 20, wherein the probe set is specific for both the detection of *Dekkera/Brettanomyces* yeast as well as other organisms of interest in the same sample[and in the same assay].

24. (Amended) The probe set of claim 23, wherein the [different] probes of the set are independently detectable.

33. (Amended) A [probe] set <u>of enzyme-linked probes</u> [suitable] for detecting, identifying or quantitating *Dekkera bruxellensis* yeast in a sample of interest.

48. (Amended) The method of claim 47, further comprising the step of:

d) growing the isolated yeast by culture in [a suitable] media.

49. (Amended) The method of claim 48, wherein the culture is grown directly on the filter, under suitable culture conditions, by placing the filter in contact with [suitable] media.

60. (Amended) A method for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample; said method comprising:

a) contacting one or more species of yeast in the sample with one or more <u>enzyme-linked</u> *Dekkera/Brettanomyces* yeast specific probes, under suitable hybridization conditions, to thereby form a probe/target sequence hybrid; and

b) detecting the presence, absence or amount of probe/target sequence hybrid and correlating the result with the presence, absence or number of *Dekkera/Brettanomyces* yeast in the sample.

61. (Amended) The method of claim 60, wherein one or more of the *Dekkera/Brettanomyces* yeast specific probes comprise[s] a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

62. (Amended) The method of claim 61, wherein the probing nucleobase sequences [are exactly as represented] of said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

72. (Amended) A kit [suitable] for performing an assay that detects, identifies or enumerates *Dekkera/Brettanomyces* yeast in a sample, wherein said kit comprises:

a) one or more enzyme-linked *Dekkera/Brettanomyces* specific probes; and

b) other reagents or compositions necessary to perform the assay.

80. (Amended) The kit of claim 72, comprising:

a) a filter for isolating yeast from a sample of interest;

b) culture media for growing the isolated yeast;

c) fixation solution for fixing grown yeast;

d) a hybridization solution suitable for imposing suitable hybridization conditions;

e) a soy bean peroxidase enzyme labeled probe specific for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in the sample;

f) one or more wash solutions for removing undesirable components after performing one or more steps of the assay; and optionally

g) an enzyme substrate suitable for generating detectable signal from [the] enzyme activity of the soy bean peroxidase enzyme linked to the [peptide nucleic acid] probe; or

h) a film for detecting signal generated from the enzyme activity.

83. (Amended) A method for quantitating slow growing yeast in a liquid sample in less than 48 hours; said method comprising:

a) filtering a fixed volume of liquid using a filter having a pore size that does not allow the yeast to pass;

b) incubating the filter containing the yeast, in [a suitable culture] media and under [suitable] culture conditions, for 45 or fewer hours to thereby grow microcolonies of yeast;

c) fixing the microcolonies of yeast to the filter;

d) contacting the microcolonies of yeast with a yeast specific enzyme-linked probe, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast;

e) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample; and

f) determining the quantity of yeast in the sample.

84. (Amended) The [kit] method of claim 83, wherein fixing the microcolonies of yeast to the filter and contacting the microcolonies of yeast with a yeast specific enzyme-linked probe are performed simultaneously using a single solution.

85. (Amended) The [kit] method of claim 83, wherein the number of CFU in the sample is determined.

(iii) Please add the following new claims:

86. (New) The probe set of claim 9, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

87. (New) The probe set of claim 20, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

(iv) Comments On The Amendments To The Claims

a. Claim 1 has been amended in view of various rejections raised by the Examiner under 35 U.S.C. §112, second paragraph.

b. Claims 5-7 have been amended in view of the rejection raised by the Examiner under 35 U.S.C. §112, second paragraph.

c. Claim 9 has been amended in view of various rejections raised by the Examiner under 35 U.S.C. §112, second paragraph. The claim has also been amended to obviate various rejections under 35 U.S.C. § 102 & 103(a).

d. Claim 11 has been amended in view of various rejections raised by the Examiner under 35 U.S.C. §112, second paragraph.

e. Claim 20 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph. The claim has also been amended to obviate various rejections under 35 U.S.C. § 102 & 103(a).

f. Claim 21 has been amended in view of an objection raised by the Examiner. It has also been amended to more distinctly claim the subject matter for which applicants seek letters patent.

g. Claim 22 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph.

h. Claim 23 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph.

i. Claim 24 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph.

j. Claim 33 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph. The claim has also been amended to obviate various rejections under 35 U.S.C. § 102 & 103(a).

k. Claims 48 and 49 have been amended in view of various rejections raised by the Examiner under 35 U.S.C. §112, second paragraph.

l. Claim 60 has been amended in view of a rejection under 35 U.S.C. §102(b).

m. Claim 61 has been amended in view of an objection raised by the Examiner. It has also been amended to more distinctly claim the subject matter for which applicants seek letters patent.

n. Claim 62 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph.

o. Claim 72 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph. Claim 72 has also been amended to obviate a rejection under 35 U.S.C. § 103(a).

p. Claim 80 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph.

q. Claim 83 has been amended in view of a rejection raised by the Examiner under 35 U.S.C. §112, second paragraph. Antecedent basis for microcolonies of yeast can be found throughout the specification but in particular in the claim itself as originally filed and in the specification at page 22, line 10 to page 23, line 28 (see in particular the opening paragraph page 22, lines 11-18).

r. Claims 84 and 85 have been amended in view of rejections raised by the Examiner under 35 U.S.C. §112, second paragraph.

s. New claims 86 and 87 have been added to more distinctly claim the subject matter for which Applicants seek letter patent.

No new matter has been added by this amendment.